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Original article

Isolation and characterization of a highly effective bacterium *Bacillus cereus* b-525k for hexavalent chromium detoxification

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ABSTRACT

The chromate resistant Gram-positive *Bacillus cereus* strain b-525k was isolated from tannery effluents, demonstrating optimal propagation at 37 °C and pH 8. The minimum inhibitory concentration (MIC) test showed that *B. cereus* b-525k can tolerate up to 32 mM Cr^{6+}, and also exhibit the ability to resist other toxic metal ions including Pb^{2+} (23 mM), As^{3+} (21 mM), Zn^{2+} (17 mM), Cd^{2+} (5 mM), Cu^{2+} (2 mM), and Ni^{2+} (3 mM) with the resistance order as $Cr^{6+} > Pb^{2+} > As^{3+} > Zn^{2+} > Cd^{2+} > Ni^{2+} > Cu^{2+}$. *B. cereus* b-525k showed maximum biosorption efficiency (q) of 51 mM Cr^{6+}/g after 6 days. Chromate stress elicited pronounced production of antioxidant enzymes such as catalase (CAT) 191%, glutathione transferase (GST) 192%, superoxide dismutase (SOD) 161%, peroxidase (POX) 199%, and ascorbate peroxidase (APOX) (154%). Within *B. cereus* b-525k, the influence of Cr^{6+} stress (2 mM) did stimulate rise in levels of GSH (907%) and non-protein thiols (541%) was measured as compared to the control (without any Cr^{6+} stress) which markedly nullifies Cr^{6+} generated oxidative stress. The pilot scale experiments utilizing original tannery effluent showed that *B. cereus* b-525k could remove 99% Cr^{6+} in 6 days, thus, it could be a potential candidate to reclaim the chromate contaminated sites.

1. Introduction

Industrial globalization and technological revolution where maintaining the high living standards, costing the environment maliciously. The emanating industrial effluents, containing high concentrations of toxic organic, metalloids, and heavy metal (lead, cadmium, and chromium) contaminants when released indiscriminately cause serious damage to the biosphere (Dixit et al., 2015; Nissim et al., 2018). Persistent and non-degradable nature aids metal pollutants to accumulate in the biosphere aggravating environmental and health issues worldwide. Thus, immediate actions are imperative to curtail toxic heavy metal pollutants concentration to acceptable limits (Dutt et al., 2015).

In the Earth's crust, chromium ranks 21st as the most abundant element i.e. concentration reaches upto 300 μg/g (Karim et al., 2020). Chromium has been added to the environment through natural processes (rock weathering, forest fires, volcanic eruptions etc.), nevertheless, anthropogenic inputs are the real culprits. Extensive industrial use of chromium in electroplating (chrome-plating), leather tanning, wood treatment, water cooling, paint and pigment manufacturing, and the subsequent Cr contaminated toxic waste discharge led to severe pollution of the soil–water system (Cheung and Gu, 2007).

As a transition metal, chromium exhibits various oxidation states thus correspondingly demonstrating different physicochemical properties, making acidic, basic or amphoteric oxides. Nevertheless, the most stable and prevalent forms are hexavalent

Abbreviations: MIC, Minimum inhibitory concentration; GST, Glutathione transferase; POX, Peroxidase; SOD, Superoxide dismutase; APOX, Ascorbate peroxidase; GSH, Glutathione; NPSHs, Non-protein thiols; PCR, Polymerase chain reaction; NCBI, National center for biotechnology information; BLAST, Basic local alignment search tool; OD, Optical density; FCBP, Fungal Culture Bank of Pakistan; FTIR, Fourier-transform infrared; EDX, Energy Dispersive X-Ray; SEM, Scanning Electron Microscopy; ml, Milliliter; mM, Millimolar.

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(Cr^{6+}) and trivalent chromium (Cr^{3+}). Hexavalent chromium is 1000 times more toxic as compared to its trivalent counterpart. Alarming concentration of Cr^{6+} compounds in the industrial effluents is largely because of its extensive utilization in various industries including chrome electroplating, leather tanning, petroleum refinery, alloy production, paint and pigment manufacturing (Cheung and Gu, 2007).

Due to high water solubility, Cr^{6+} compounds easily gain access to the food chain that aids in rapid mobility and easily traversing the biological membranes that ultimately damage the intracellular nucleic acids and proteins. The U.S. Environmental Protection Agency (EPA) recognized Cr^{6+} not only as the priority pollutant but also placed it under Group 'A' human carcinogen category (Dhal et al., 2013). In contrast, Cr^{3+} being comparatively benign, rapidly forms insoluble precipitates such as oxides and hydroxides at neutral pH (Jeyasingh and Philip, 2005; Dhal et al., 2013).

Currently, to detoxify chromate polluted environment, various conventional physicochemical methods could be utilized such as soil washing, land filling, physicochemical extraction etc., however, all the procedures demand high chemical inputs, making them quite expensive and cumbersome with drawbacks of sludge production (Jeyasingh and Philip, 2005). In this regard, bioremediation offers an inexpensive, innovative and eco-friendly approach to detoxify metal contaminated environment with the help of indigenous microorganisms (Akcil et al., 2015). Native microbiota in the heavy metal polluted wastewater have established the strategies for their survival to fight off the metal generated hazards by various methods such as metal adsorption, absorption, oxidation, reduction and methylation (Mangaiyarkarasi and Geetharamani, 2014; Liu et al., 2020).

The bacterial strains which have been endowed with the ability to reduce chromate are regarded as chromium-reducing bacteria (CRB), which could be easily found in the effluents discharged by the industries, especially from textile, tanneries, and chrome electroplating manufacturing as well as the soil polluted with these effluents (Elahi and Rehman, 2019a). The chromate resistance strains belongs to *Staphylococcus, Microbacterium*, *Ochrobactrum*, *Bacillus* and *Brevibacterium* have already been reported previously in Cr^{6+} reduction and bioremediation scenario (Zahoor and Rehman, 2009; Mangaiyarkarasi and Geetharamani, 2014; Akcil et al., 2015; Elahi and Rehman, 2019; Li et al., 2020; Li et al., 2021).

The current study describes the detoxification potential of the Cr^{6+} resistant gram-positive strains *B. cereus* b-525k in the original tannery effluent. To determine the route of transportation of hexavalent chromium ions and its detrimental effects within the cell, Fourier-transform infrared, Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray (EDX) analysis and antioxidant enzymes profiling were also performed.

2. Materials and methods

2.1. Isolation of Cr^{6+} resistant bacteria

To isolate Cr^{6+} resistant bacterial strains, tannery sewage wastewater was obtained from a long-term chromate polluted industrial sites of Qasoor, and Sheikhupura, Punjab (Pakistan). The samples were subjected to serial dilution prior to spreading on the LB agar plates containing 1 mM chromate stress (i.e. $K_2Cr_2O_7$) and plates were incubated at 37 °C for 72 h till the growth appears. Final selection was done on the ability of bacterial strains to resist as well as reduce high concentrations of Cr^{6+}.

Selected bacterial strain b-525k was purified and characterized by routine biochemical tests according to Cappuccino and Sherman (2008) while the molecular characterization was performed by Masneuf-Pomarède et al. (2007). Briefly, genomic DNA of b-525k was isolated, which was then subjected to 16S rRNA gene amplification through primers (Rehman et al., 2007). The amplified products were mailed for sequencing to Macrogen, Korea and the sequence obtained was then aligned by utilizing BLAST analysis. Moreover, a dendrogram was created by using MEGA7 program on the basis of homology.

2.2. Determination of optimal cultivation parameters

Optimal cultivation parameters were ascertained. For optimal growth temperature, bacterial strain b-525k was cultivated in 250 ml flask containing 100 ml LB broth and bacterial cells were cultured at different temperatures i.e. 20, 30, 37, and 50 °C, for a time period of twenty-four hours. Cell densities were measured at $OD_{600\ nm}$. For optimum pH, LB broth was prepared with different pH values (i.e. 5, 6, 7, 8, 9, and 10); autoclaved and inoculated with the 24 h old b-525k cells. The flasks were shifted to a shaking incubator at 37 °C, 130 rpm for 24 h, and cell growth was measured at $OD_{600\ nm}$.

Growth profile of b-525k was ascertained with and without chromate (Cr^{6+}) stress. The bacterial strain was grown in mineral salt medium (MSM) broth with no metal (control), and supplemented with 2 mM $K_2Cr_2O_7$ (treated). The cell density of the growth curve was estimated by taking absorbance at 600 nm after regular intervals up to 24 h. The experiment was performed in thrice.

2.3. Evaluation of minimum inhibitory concentrations (MICs)

To determine MICs, various metal salts were employed such as $K_2Cr_2O_7$ (for Cr^{6+}), $CuSO_4{\cdot}5H_2O$, $CdCl_2$, $PbNO_3$, $NiCl_2{\cdot}6H_2O$ and $ZnSO_4{\cdot}7H_2O$. Heavy metals concentrations were added separately to 100 ml slightly modified M9 broth medium and log phase culture of b-525k was used for inoculation. All the flasks were incubated under shaking conditions at 37 °C at 130 rpm for 7 days. The cell densities of culture were measured by taking $OD_{600\ nm}$. The minimum concentration of metal which could hamper growth of bacterial cells was regarded as MIC.

2.4. Enzymes and glutathione contents determination

Under the influence of Cr^{6+} stress, any change in the antioxidant enzymes profile of b-525k was studied to understand the effect of metal stress within the cell. For this, b-525k bacterial strain was cultivated in 100 ml MSM media under shaking conditions at 37 °C, with or without Cr^{6+} stress. As control, b-525k was cultivated without any Cr^{6+} stress for 48 h while in case of treated, b-525k was first cultured for 24 h and then Cr^{6+} (2 mM) was supplemented in the culture medium and the flask was again shifted to incubator at 37 °C for another 24 h. After incubation, growth was collected by centrifugation at 14,000 rpm for 10 min, supernatants were discarded while the weighted pellets were resuspended in phosphate buffer before sonication. The collected aliquots, achieved through centrifugation of sonicated cell pellets, were used to measure the antioxidant enzymes activities. Glutathione transferase (GST) assay was performed as the protocol described in Habig et al. (1974) while the protocol of Reuveni et al. (1992) was used with slight modifications to assay peroxidase (POX) enzyme. The enzymatic activities of catalase (CAT) and superoxide dismutase (SOD) were assayed according to Beers and Sizer (1952) and Ewing and Janero (1995), respectively, and for ascorbate peroxidase (APOX) procedure of Israr et al. (2006) was followed.

Effect of chromate on the induction of glutathione (GSH) and non-protein thiols (NPSHs) was assayed according to procedure described in Khan et al. (2015). For this, MSM medium was prepared in three flasks, inoculated with log phase b-525k culture

and incubated at 37 °C for 24 h. Then, $K_2Cr_2O_7$ (2 mM) stress was added in 2 flasks, while the 3rd flask without metal acted as control. These flasks were again shifted to the incubator at 37 °C for another 48 h. Then, cells were harvested through centrifugation at 14,000 rpm for 10 min, and washed with 1 mM phosphate buffer. The washed cells were weighed and re-suspended in 1 ml of 5% sulfosalicylic acid. Bacterial cells were then sonicated, centrifuged and the resulting supernatant was equally segregated into two portions. First portion was utilized to measure GSH amount and the second portion was utilized to determine NPSHs amounts (Khan et al., 2015).

2.5. Determination of metal processing ability of b-525k

To evaluate metal processing potential of b-525k, any alteration in Cr^{6+} quantity was measured through Atomic Absorption Spectrophotometer as described by Rehman et al. (2010). For this three flasks were prepared in which two flasks of bacterial culture b-525k supplemented with 2 mM Cr^{6+} stress (treated) and one flask with only 2 mM Cr^{6+} metal stress but no organism (control), and all the flasks were incubated at 37 °C, 130 rpm. Five ml portion was withdrawn from each flask after a suitable time period (i.e., 2, 4, 6, 8 days), and growth was harvested through centrifugation at 6,000 rpm for a period of 10 min. The resulting supernatant and pellet were evaluated for Cr^{6+} concentration by Rehman et al. (2010).

2.6. Bacterial reduction potential of hexavalent chromium

Hexavalent chromium reduction ability of b-525k was studied in original tannery wastewater. For this, three 20 L plastic containers were utilized in which two acted as control; the 1st control consisted of only 10 L real tannery wastewater while the 2nd control contained 10 L distilled water along with 1.5 L fresh b-525k culture, and the third container had the treated wastewater (10 L tannery wastewater inoculated with 1.5 L fresh b-525k culture). Then, containers were augmented with hexavalent chromium stress (2 mM) and placed at room temperature (25 ± 2 °C). After a regular time period of 2, 4, 6, and 8 days of incubation, 10 ml sample was removed from every container, centrifuged at 4000 rpm for 10 min and the resulted supernatants were subjected to Diphenylcarbazide method to evaluate residual Cr^{6+} concentration. Any change in the Cr^{6+} concentration was measured through an already prepared calibration curve. The experiment was performed thrice.

2.7. Fourier-transform infrared spectroscopy (FTIR) and SEM/EDX characterization

Fourier-transform infrared (Bruker, alpha) analysis provides the information regarding the functional groups' involvement under normal and Cr^{6+} stress conditions and how the metal ions impact the changes on the surface of the bacteria. Deokar et al. (2013) protocol was used to prepare samples for FTIR analysis. In the course of metal-microbe interaction, FTIR analysis also provides information of the location of hexavalent or trivalent chromium ions on the cell surface. Intracellular uptake of chromium metal ions was confirmed with Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray (EDX) analysis. For SEM-EDX analysis, specimens were prepared according to procedure described in treated as me Khan et al. (2016).

2.8. Data analysis

For each experiment at least three separate flasks were maintained. Each time three readings were taken, their mean, and standard error of the mean were calculated.

3. Results

3.1. Isolation and screening of hexavalent chromium resistant bacteria

In order to obtain chromate resistant bacteria, tannery wastewater samples were diluted before spreading on to the LB agar plates with added chromate stress (2 mM Cr^{6+}). The criterion for an ideal candidate was to choose the one which not only resists high Cr^{6+} concentration but is also capable of reducing it into its trivalent form. For this purpose, the concentration of chromate stress was increased gradually till MIC was achieved. Selected bacterial strain, b-525k, was gram positive rod and attributes of the strain are given in table S1, could resist Cr^{6+} upto 32 mM. The 16S ribotyping revealed that it exhibited 100% homology with *Bacillus cereus* with accession number KX941838, already submitted to the NCBI database. Figure S1 is showing a dendrogram made on the similarity basis with strains of other *B. cereus* through the MEGA7 program using 500 bootstrap values. *Bacillus cereus* strain b-525k has also been deposited at First Fungal Culture Bank of Pakistan under accession number of FCBP-B-734.

3.2. Optimum cultivation parameters and cross metal resistance

Optimum cultivation of *B. cereus* b-525k was observed at pH 8 when incubated at 37 °C however, the presence of Cr^{6+} stress has substantially declined the growth pattern (Fig. 1). *B. cereus* b-525k could resist not only Cr^{6+} (32 mM) but also some other heavy metals tested, i.e., Pb^{2+} (23 mM), As^{3+} (21 mM), Zn^{2+} (17 mM), Cd^{2+} (5 mM), Cu^{2+} (2 mM), and Ni^{2+} (3 mM) with the resistance order as Cr^{6+} >Pb^{2+} >As^{3+} >Zn^{2+} >Cd^{2+} >Ni^{2+} >Cu^{2+}.

3.3. Quantification of antioxidants and glutathione

Study of antioxidants of *B. cereus* b-525k, with and without chromate stress, revealed that as compared to control, Cr^{6+} presence has provoked significant surge in production of all antioxidants including CAT (191%), GST (192%), SOD (161%), POX (199%) and APOX (154%) (Fig. 2). Within *B. cereus* b-525k, the influence of Cr^{6+} stress (2 mM) did stimulate rise in levels of GSH, GSSG

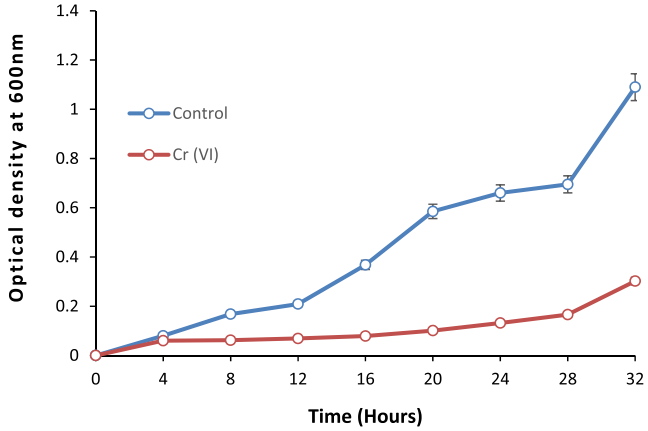

Fig. 1. Growth curve of *B. cereus* b-525k in MSM without Cr^{+6} (control) and MSM augmented with 2 mM Cr^{+6} (treated) incubated at 37 °C. OD_{600nm} was measured after regular time interval. Each value is the mean of three readings (n = 3).

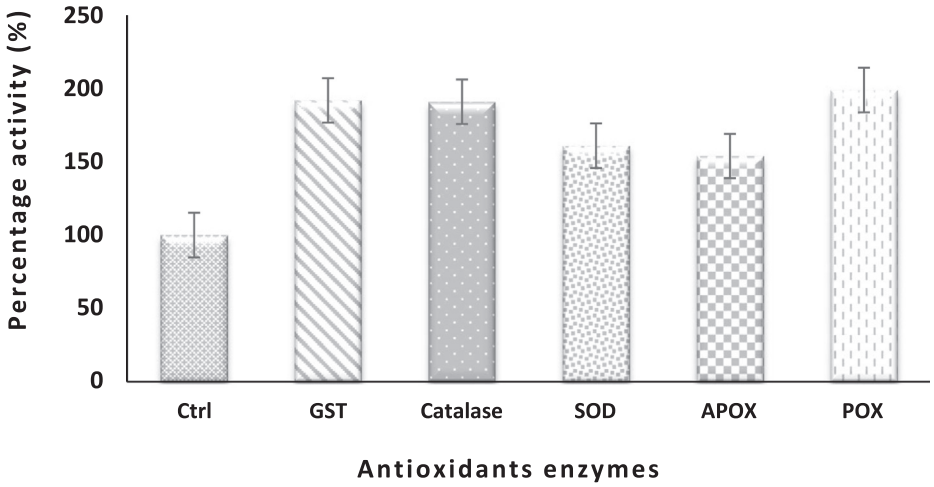

Fig. 2. Alteration in activities of antioxidant enzymes, as exhibited by *B. cereus* b-525k under 2 mM Cr^{6+} stress Each value is the mean of three readings (n = 3).

Table 1
Levels of reduced (GSH) and oxidized glutathione (GSSG), total glutathione, reduced and oxidized glutathione ratio, and non-protein thiols in *B. cereus* b-525k under 2 mM Cr^{6+} stress.

Cr Conc. (mM)	GSH (mMg^{-1} FW)	GSSG (mMg^{-1} FW)	GSH + GSSG (mMg^{-1} FW)	GSH/GSSG ratio	% increase in GSH	Non-protein thiols	% increase in non-protein thiols
0.0	13.605	3.023	16.629	4.500	907.03	15.369	541.7
2.0	22.676	13.228	35.903	1.714		20.786	

and non-protein thiols (Table 1), i.e. rise in GSH (907%) and non-protein thiols (541%) was measured as compared to the control (without any Cr^{6+} stress).

3.4. Assessment of chromium processing potential

To determine the biosorption potential, *B. cereus* b-525k was cultivated in LB broth containing 2 mM Cr^{6+} stress (Fig. 3). The biosorption efficiency (q) of bacterium was 22, 32, 51, and 29 mM Cr^{6+}/g after 2, 4, 6 and 8 days of incubation. The concentration of Cr^{6+} absorbed (uptake) by *B. cereus* b-525k cells after 2, 4, 6 and 8 days was 6, 7, 13, and 6 mM/g, respectively while 5, 7, 13, and 6 mM/g of Cr^{6+} were determined to be adsorbed on the cell surface of *B. cereus* b-525k after the incubation of same time duration.

3.5. Cr $^{6+}$ removal

The capability of *B. cereus* b-525k to convert Cr^{6+} into Cr^{3+} was measured at pilot scale, where potential of the bacterium was ascertained in 10 L tannery industrial wastewater and reduction of Cr^{6+} into Cr^{3+} was determined through by Diphenylcarbazide method. The reduction results indicated that strain b-525k was able to reduce upto 99% Cr^{6+} into Cr^{3+} from the tannery industrial effluent within a time period of 6 days (Table 2) when concentration of hexavalent chromium was kept at 2 mM (Fig. S2a,b).

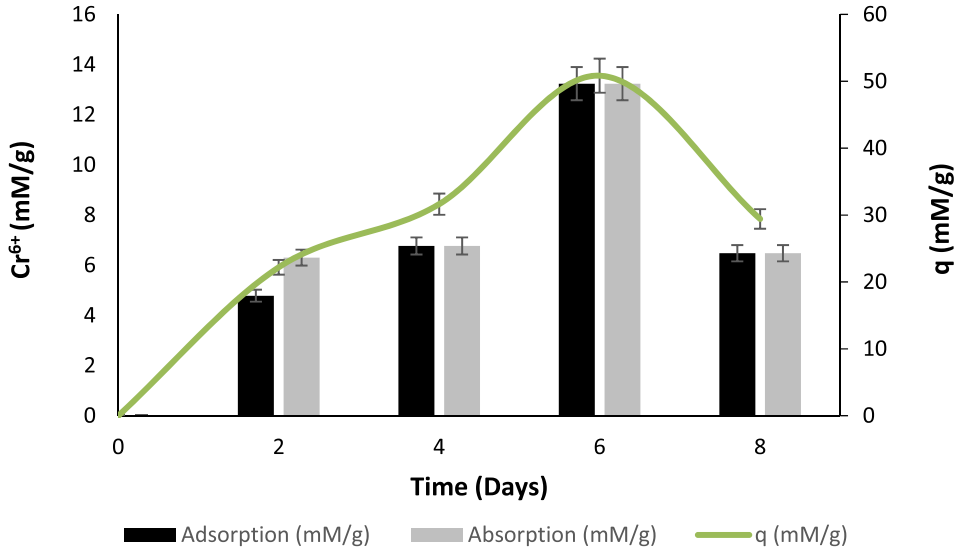

Fig. 3. Metal (Cr^{6+}) processing potential exhibited by *B. cereus* b-525k, measured at lab scale. Each value is the mean of three readings (n = 3).

Table 2
After the treatment with *B. cereus* b-525k, determination of change in Cr^{6+} (μM) concentration commensurate with the percentage reduction after regular intervals (2, 4, 6, 8 days) when the original tannery sewage water was augmented with 2 mM Cr^{6+} stress.

Bacterial strain	Days	2	4	6	8
B. cereus b-525k (Water)	Cr^{6+} (μM)	15.58	13.85	0.08	0.08
	% Reduction	22	31	99	99
B. cereus b-525k (Effluent)	Cr^{6+} (μM)	5.30	0.6	0.42	0.19
	% Reduction	73	97	98	99

3.6. Fourier-transform infrared spectroscopy (FTIR) and SEM/EDX characterization

FTIR analysis of *B. cereus* b-525k with and without Cr^{6+} has been shown in figure 5. FT-Infrared spectra of bacterium, without Cr^{6+} stress, showed specific absorption peaks of amino, hydroxyl, carboxyl, and sulfonate groups which exhibited corresponding group presence on the bacterial cell surfaces. However, when the bacterial cells were challenged to Cr^{6+} stress, alterations in peaks in the range of 3275 cm^{-1} and 1800–1000 cm^{-1} were determined. FTIR analysis revealed the shifting of various peaks of *B. cereus* b-525k in metal-treated culture from 1633 to 1628, 1535 to 1526, and 1052 to 1057. A minor change in hydroxyl group area was also noted (Fig. 4a). Accumulation of Cr^{6+} within the cells of *B. cereus* b-525k was determined through EDX analysis. SEM analysis showed changes in cell morphology after being challenged with 2 mM Cr^{6+}. Both energy dispersive X-ray analysis (Fig. 4b) and SEM analysis (Fig. 4c) confirm the manifest of intracellular uptake of the hexavalent chromium.

4. Discussion

In Punjab, Qasoor and Sheikhupura are among the largest industrial districts, encompassing various important industries,

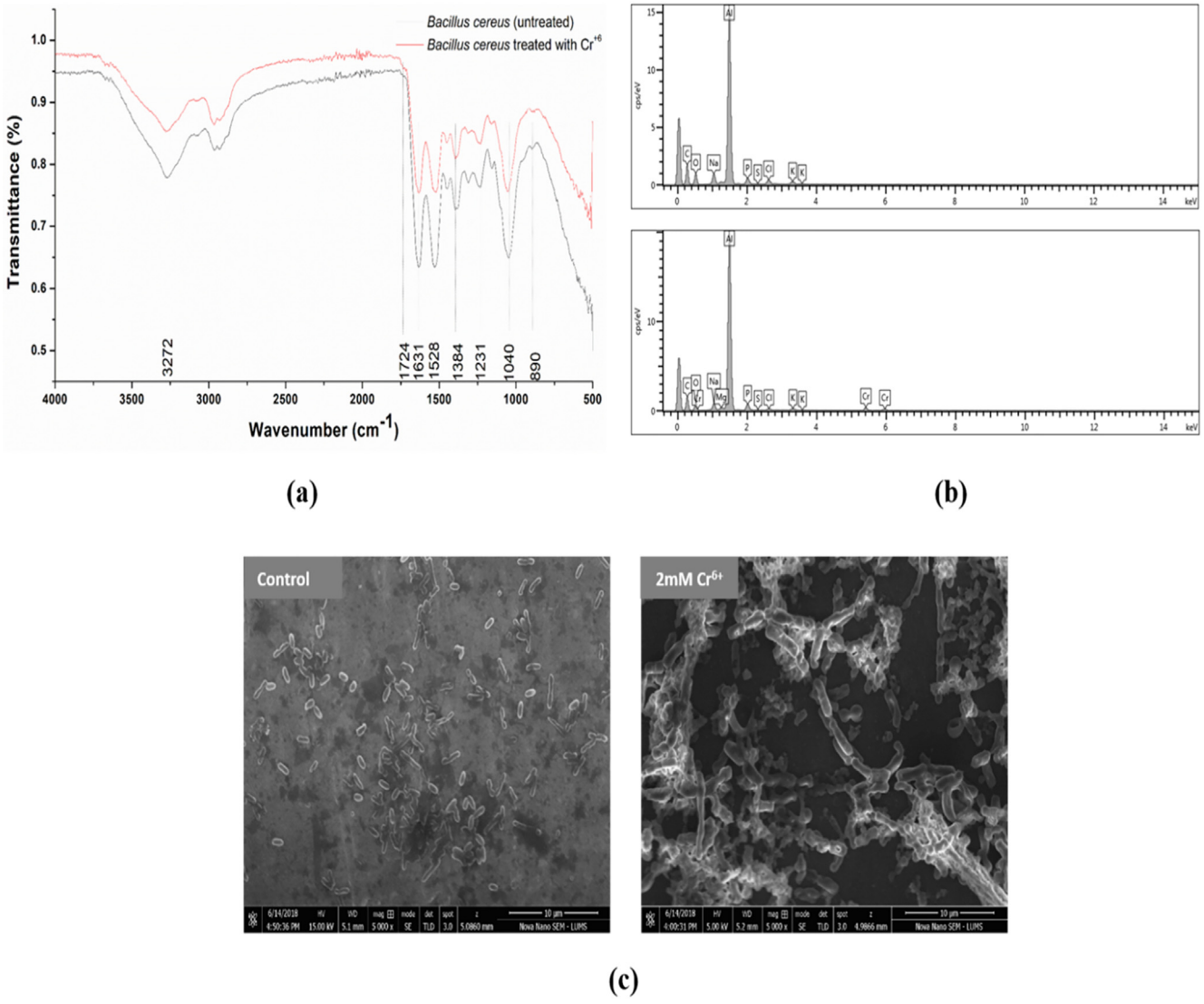

Fig. 4. (a) Fourier-transform infrared spectroscopy (b) Scanning electron microscope (c) Energy dispersive X-ray spectroscopy analysis of *B. cereus* b-525k in the absence (control) and presence of 2 mM Cr^{6+} stress.

which strengthened the economy, yet polluting the pristine environment aggressively. The industries tend to produce tons of toxic heavy metal byproducts, which when released directly into the biosphere not only damage it immediately but also have a cumulative effect owing to the non-degradable nature of the heavy metals. Thus, heavy metals tend to accumulate to alarming concentrations which then pose serious threats to the biosphere and biota equally throughout the world (Thatoi et al., 2014; Ayangbenro and Babalola, 2017).

Chromate is highly toxic as well as carcinogenic in nature, thus rising concentrations of Cr^{6+} byproducts jeopardize all forms of life. The non-degradable nature of chromate left scientists with no other choice than its biotransformation into relatively innocuous form. Thus, bioconversion of chromate (Cr^{6+}) into relatively less toxic trivalent form (Cr^{3+}) via chromate-resistant and reducing bacteria present economical and eco-friendly way to cut its concentration to the accepted level (<0.05 mgL^{-1}), as recommended by US-EPA (Mishra et al., 2012).

Bioremediation of lethal metal ions present in the industrial wastewater has become the most convenient technique for the environmental researchers owing to its advantages over the chemical procedures. Chromate resistant bacteria are not only present in the chromate polluted tannery effluents, soil, and sludge, but also exist in non-contaminated environs. Thus, the ubiquitous nature of chromate resistant strains makes them the ideal candidate to be utilized for *in situ* bioremediation (Wani et al., 2007; He et al., 2010). Nevertheless, the efficacy of the procedure could be limited by the presence of the concomitant pollutants (Turick et al., 1996). A large number of studies have been performed to figure out the capability of Cr^{6+} resistant and reducing bacterial strains in terms of bioremediation (Zahoor and Rehman, 2009; Zhang et al., 2013; Elahi et al., 2019) in aerobic and anaerobic environments (Wani et al., 2007; Elahi and Rehman, 2019a; Chen et al., 2021).

In this research work, 13 Cr^{6+} resistant strains were isolated from long term chromate polluted industrial sites of Qasoor, and Sheikhupura, out of which *B. cereus* b-525k was selected for further studies. Chromate resistant *Bacillus* strains have been isolated and utilized by various scientists previously, whose studies demonstrated that *Bacillus* do have bioremediation potential to detoxify Cr^{6+} polluted sites via *Bacillus megaterium* TKW3, *B. methylotrophicus, B. subtilis*, *B. circulans*, and *B. cereus* (Cheunga and Gu, 2007; He et al., 2010; Mangaiyarkarasi and Geetharamani, 2014; Singh et al., 2013; Mala et al., 2014; Li et al., 2020).

Presence of chromate ions within the cell compel the microorganisms to adopt different strategies for their own survival, thus only Cr^{6+} resistant microbes could resist, utilizing metal in trace amounts for the functioning of metabolism while resist and/or detoxify its excessive amount. Microbes differ in their potential to uptake and process the Cr^{6+} ions through biosorption, bioaccumulation, and chromate effluxion and/or reduction. Chromate resistance and reduction are two independent phenomena, meaning that a microbe able to resist chromate may not necessarily reduce it also. Chromate reduction and its biotransformation into trivalent form have been far more extensively studied in bacteria as compared to yeasts, fungi and actinomycetes. Reduction potential of a bacterial strain to reduce chromate is directly or indirectly depending on several factors such as the level (concentration) of Cr^{6+} toxicity as well as occurrence of other heavy metal ions in the effluent samples (Mala et al., 2014).

Generally, a potential candidate detoxifies chromate ions in aqueous medium by utilizing mechanisms of biosorption and/or bioaccumulation. In the first step, chromate ions just passively adhere to the bacterial surface which then converts into chemical bondage on the corresponding affinity sites leading to chromate reduction into Cr^{3+}. It should be noted that chromate ions (Cr^{6+}) do not have any specific influx channel, however, they gain entry in the cell by exploiting sulphate and phosphate channels, owing to the structural similarity with the sulphate / phosphate ions (Cervantes and Campos-García, 2007; Mala et al., 2014; Baaziz et al., 2017). Under aerobic conditions, chromate reduction into Cr^{3+} is not a pretty straightforward conversion rather it provoke generation of several toxic transient Cr (Cr^{4+}, Cr^{5+}) and ROS species which in turn cause significant damage at cell and molecular level (Bharagava and Mishra, 2017).

Metal generated ROS production alter the reduced environment within the cell, however, this alteration in homeostasis could be prevented through the activities confer by antioxidant enzymes (catalase, glutathione transferase, and superoxide dismutase) by converting noxious reactive oxygen species in to harmless compounds (Hussein and Joo, 2013). Antioxidant profiles of *B. cereus* b-525k with and without 2 mM Cr^{6+} stress demonstrated that metal stress does elevate expression of all the antioxidants, particularly POX where 99% rise in levels were observed. Peroxidases are also regarded as stress enzymes, because various environmental stresses could provoke their production such as in cases of metal ions stress (Cd, Al, Zn, and Cu), drought, water and gamma-radiation stress (Hussein and Joo, 2013). Present study results are in good agreement with the findings of Suthar et al. (2014), who also noted that hexavalent chromium stress induces profound increase in all of the antioxidant enzymes. Glutathione and non-protein thiols play an important role in combating the ROS toxicity (Elahi and Rehman, 2019). Newton et al. (2009) reported that bacillithiol (BSH), a thiol compound found in most of the Bacillus species, is likely involved in maintaining cellular redox balance and plays a role in microbial resistance to various antibiotics.

Wastewater provides a quite inhospitable habitat for the cultivation of non-native bacteria due to lack of proper nutrients essential for the growth of microorganisms, plus carrying toxic compounds. A lot of research has been done on evaluating the chromate reduction in LB broth media, (He et al., 2010; Das et al., 2014), nevertheless, only few reproduce the bioreduction trials in the real industrial effluents. In this study we have tested the ability of the strain in LB and MSM medium as well as we have replicated the bioremediation trials at pilot scale level using the original tannery effluent, demonstrating that these bacterial strains have the potency to reduce Cr^{6+} from original tannery wastewater as well. Thus, these strains are the potential candidate to be utilized in bioremediation trials in real time scenarios. Zahoor and Rehman (2009) also replicated the chromate bioreduction potential of *Staphylococcus capitis* and *Bacillus* sp. JDM-2-1 in the original industrial wastewater demonstrated that both organisms have the potential of reducing Cr^{6+} upto 89% and 86%, respectively, where the initial concentration was 100 mg/L.

To confirm the course of Cr^{6+} adsorption, absorption and reduction, FTIR/SEM-EDX analyses were performed. Functional groups present on the surface of bacterial cells play a crucial role in binding with metal ions, and their subsequent processing. FTIR analysis of control (without Cr^{6+} stress) *B. cereus* b-525k revealed the adsorption peaks corresponding to the presence of various functional groups such as hydroxyl groups, CO, –NH groups moieties. However, under influence of Cr^{6+} stress, changes in adsorption peaks as well as in the intensities of these peaks were observed (Fig. 4a). This may be due to interaction of Cr^{6+} ions with the functional groups. SEM/EDX analysis also revealed alteration of the cell surface i.e., wrinkled (treated) as compared to the smooth (untreated) surface and presence of both Cr^{6+} and Cr^{3+} within the cell, confirmed the conversion (i.e. reduction) of Cr^{6+} into Cr^{3+}. The results of the present study are in good agreement with the findings of Lameiras et al. (2008), Pandi et al. (2009), Das et al. (2014), and Khan et al. (2016).

5. Conclusion

In conclusion, chromate resistant Gram-positive *B. cereus* strain b-525k isolated from tannery effluents, was demonstrating optimal growth at 37 °C and pH 8. The MIC experiment showed that *B. cereus* b-525k can tolerate up to 32 mM Cr^{6+}, and also exhibit the ability to resist other toxic heavy metals. *B. cereus* b-525k showed maximum biosorption efficiency (q) of 51 mM Cr^{6+}/g after 6 days. Chromate stress elicited pronounced production of antioxidants such as SOD, CAT, APOX, POX, and GST in *B. cereus* b-525k. Moreover, elevated production of glutathione as well as other non-protein thiols were detected which markedly nullifies Cr^{6+} generated oxidative stress. The pilot scale experiments utilizing original tannery effluent showed that *B. cereus* b-525k could remove 99% Cr^{6+} in 6 days. *B. cereus* b-525k could be a potential candidate to eradicate the toxic chromate from the metal contaminated sites.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2022.01.027.

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